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October 24, 1997

Dr. Larry G. Hart
Executive Secretary
National Toxicology Program
111 Alexander Drive
Building 101
Research Triangle Park, NC 27709

Re: Review of Substances for Listing in the NTP's Ninth *Biennial Report on Carcinogenicity*

Dear Dr. Hart:

Zeneca Pharmaceuticals, a business unit of Zeneca Inc., wishes to make an oral presentation at the National Toxicology Program (NTP) Board of Scientific Counselors' Meeting to be held October 30 & 31 and hereby respectfully submits the enclosed written materials in response to the proposal to list tamoxifen as a substance "known to be a human carcinogen" in the Ninth *Biennial Report on Carcinogens.*

Zeneca believes that tamoxifen has not been shown to cause endometrial cancer, the conclusion reached by an International Agency for Research on Cancer (IARC) working group "that there is sufficient evidence in humans for the carcinogenicity of tamoxifen in increasing the risk for endometrial cancer" notwithstanding. We believe that the genotoxic mechanisms which have been proposed to account for the development of hepatocarcinomas in rats are not relevant to the human situation. In addition, we believe that the epidemiologic studies cited by NTP in the Draft RC Background Document for Tamoxifen (draft RC document) as justification for listing tamoxifen as an agent "known to be a human carcinogen" do suffer from confounding factors and bias, and that this could affect the reported results. A number of eminent physicians and scientists have reviewed this issue and concluded that there is insufficient evidence to conclude that tamoxifen causes endometrial cancer.

The studies cited by NTP are already known to the U.S. Food and Drug Administration (FDA) and to the National Cancer Institute (NCI). Appropriate actions to protect the public health have already been taken. Information on the association of tamoxifen and endometrial cancer in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14, being conducted under the auspices of the NCI, and Stockholm trials has been included in the prescribing information for tamoxifen since 1993. It is also included in our informational materials to physicians and other health care providers. A listing of tamoxifen as a carcinogen has the potential to disrupt communication between patients and their physicians. Feedback Zeneca has received from patient groups suggests that following the listing of tamoxifen as a carcinogen by the State of California many patients stopped taking tamoxifen without telling their physicians.

Listing of tamoxifen as a carcinogen by the NTP will not protect the public health; indeed to the extent patients with breast cancer stop taking tamoxifen as a result of a listing, public health will be jeopardized. It should be noted that tamoxifen is the only antiestrogen cleared by the FDA for adjuvant therapy of breast cancer, and is the only antiestrogen cleared for use in premenopausal women.

Following the IARC decision, information from a case-control study conducted by Dr. Leslie Bernstein of the University of Southern California became available. The study confirms the important role of the confounding factors of obesity and prior use of unopposed estrogen replacement therapy. Publication is expected in the next few months.

In addition, the NSABP P-1 trial (the "Prevention Trial") required baseline endometrial sampling on all participants who entered the trial after July 8, 1994, and yearly gynecologic evaluations after September 1994. This trial has recently met its recruitment goal, and should be able to clarify any relationship between tamoxifen and endometrial carcinoma. Definitive results are expected in around 2 years. Although we realize that NTP relies only upon evidence of carcinogenicity that has been peer reviewed, we think it appropriate that members of the Board be aware of the state of current research, which is likely to yield data critical to the issues presented by the proposed listing.

In view of the above, Zeneca submits that it would be inappropriate for the Board to recommend that tamoxifen be listed as a substance known to cause cancer. In support of that position, we enclose the following:

- commentary of Zeneca Pharmaceuticals to the Draft NTP RC Background Document on Tamoxifen
- summary of oral presentation I intend to make at the meeting of the NTP Board of Scientific Counselors
- *Seminars in Oncology*, "Scientific Review of Tamoxifen."

Respectfully submitted,

Mark Steinberg, M.D.
Associate Director, Oncology
Medical Research and Communication Group

Enclosures

My name is Mark Steinberg, and I am speaking on behalf of Zeneca Pharmaceuticals, discoverer and developer of tamoxifen, the most widely prescribed breast cancer treatment in the world. I would like to thank the Board for giving us this opportunity to present new information on tamoxifen.

I would like to begin with two points I think we can all agree on. First, the data do not support an association of tamoxifen with primary liver cancers in humans. Second, the endometrial cancers seen in women who have taken tamoxifen have a similar stage, grade and prognosis to those seen in the general population.

Now, it is one thing to recognize an association between tamoxifen and endometrial cancers; it is another to ascribe causation. What appears to be a logical cause-and-effect relationship may actually have another explanation. As you know, a working group of IARC, the International Agency for Research on Cancer, reviewed the literature on tamoxifen in February 1996. All of the clinical studies reviewed by the working group were based on retrospective reviews of numbers of cases of endometrial cancer. Even at that time, Zeneca felt that while some studies had reported an association between tamoxifen and endometrial cancer, many variables, including possible confounding factors and detection bias might have affected the study results. Many eminent scientists outside Zeneca agreed. It is only since that time that data from studies specifically designed to avoid those deficiencies is starting to emerge.

Dr. Leslie Bernstein, Professor of Preventive Medicine at the University of Southern California, has completed a case-control study of endometrial cancers in breast cancer patients. She has identified obesity and prior used of unopposed estrogens for hormone replacement therapy as powerful confounding factors. Now, when she looked at tamoxifen and endometrial cancer without regard to confounding factors, her study did reveal a statistically significant association between tamoxifen and endometrial cancer. However, when she eliminated from the analysis the data from women who had received unopposed estrogen, the association virtually disappeared. This study suggests that the judgment of the working group that confounding factors were unlikely to affect the results of the studies it had reviewed was in fact

incorrect. Further, it gives a plausible explanation (prior use of unopposed estrogens) for the results seen in some earlier studies. This work was presented at the annual meeting of the National Surgical Adjuvant Breast and Bowel Project (NSABP) in Los Angeles last June, and to the International Society of Pharmacoepidemiology in August. Preliminary, partial results have appeared in abstract form. Dr. Bernstein has given me permission to present portions of these data at this meeting.

Even more information will be available within the next 2-3 months. Last month, the NSABP P-1 trial (otherwise known as the "Prevention Trial") finished enrolling patients having reached its recruitment goal. This trial, done under the auspices of the National Cancer Institute, is evaluating tamoxifen as a preventive agent in women at high risk for developing breast cancer. Since July 1994, all women entering the trial have been required to have baseline endometrial sampling to evaluate for occult carcinomas. Since September 1994, annual gynecologic evaluations, which include endometrial sampling, are done on all women in the trial. This trial, results from which are expected in about 2 years, will give us for the first time prospective information on tamoxifen and the development of endometrial cancer in women

The public health is protected by the product labeling for tamoxifen which has included the reported association of tamoxifen and endometrial cancer since 1993. This labeling was approved by the Food and Drug Administration, which has jurisdiction over pharmaceuticals. Feedback we have received from patient advocate groups indicates that when tamoxifen was listed as a carcinogen by the State of California and by IARC many women stopped taking tamoxifen without consulting their physicians. To list tamoxifen as a carcinogen interferes with the patient-physician communication, so necessary to a good clinical outcome, and usurps the role of physician as a learned intermediary in the patient-physician relationship. Numerous clinical studies attest that tamoxifen saves lives. A listing by the National Toxicology Program will adversely affect public health, rather than protect it.

The National Toxicology Program recently revised its role to include taking account of emerging data, and assessing the public health impact of its actions. In light of the new Bernstein data and additional

emerging data from new studies on tamoxifen, Zeneca encourages the Board not to list tamoxifen as a substance known to cause cancer as we strongly believe it would be inappropriate in view of the new data.

Comments of Zeneca Pharmaceuticals to the Draft RC Background Document for Tamoxifen

Carcinogenicity

Although some studies have reported an association between tamoxifen use and development of endometrial cancer, a causal relationship is still open to question. In its monograph, a working group of the International Agency for Research on Cancer (IARC) stated there was sufficient evidence that tamoxifen increased the risk of endometrial cancer in humans. (IARC, 1996). Zeneca along with independent experts believes that the studies cited by IARC and again by the National Toxicology Program (NTP) did not adequately address potential confounding factors, and that these confounding factors may have had a major impact on the results.

Of four case control studies mentioned in the draft RC document, only three showed an increase in endometrial cancer in women who had taken tamoxifen. One (Cook, et al., 1995) actually showed a decrease in endometrial cancer in women who had taken tamoxifen. Although it has been suggested that this may be due to the shorter duration of tamoxifen therapy, another explanation is the particular care taken in this study to address potential confounding factors such as weight, hormone replacement therapy, and presence or absence of an intact uterus (MacMahon, 1997). It lends weight to the suggestion that confounding factors may have played a role in the association of tamoxifen and endometrial cancer seen in other studies. The study of Hardell exhibited selection biases in the control group. (MacMahon, 1997). Another study (Sasco, et al., 1996) showed a strong odds ratio associated with induction of menopause by radiation therapy to the breast, raising questions of information bias. (MacMahon, 1997).

Of 14 randomized clinical trials mentioned by IARC, only 2 (Fisher, et al., 1994; Rutqvist et al 1995) showed a statistically significant increase in endometrial cancers in the tamoxifen-treated women. It is noteworthy that in both the Fisher and Rutqvist trials, the number of cases of endometrial cancer in the control group was unexpectedly low. (MacMahon, 1997). It is the use of these unexpectedly low numbers in the control group that resulted in the elevated odds ratios of 5.6 (Rutqvist, et al., 1995) and 7.5 (Fisher, et al., 1994). Fisher in fact recognized this fact and recalculated the data using a more appropriate comparison population, resulting in a relative risk estimate slightly over 2.

In 5 other trials for which an odds ratio was given, the odds ratio included 1, indicating that the result did not reach statistical significance. The simple combining of endometrial cancer cases in the 12 smaller trials, as is done in the NTP draft document, is not statistically sound as it ignores the fact that tamoxifen-treated patients tend to live longer than control patients, that several trials had more than one arm with tamoxifen, and numerous other differences in methodology between trials.

One case series (Magriples, et al., 1993) has reported that endometrial cancers associated with tamoxifen therapy are higher grade and carry a worse prognosis than those seen in patients without tamoxifen use. However, as noted in the NTP draft RC document, several other studies indicate that endometrial cancer associated with tamoxifen use has a prognosis similar to that seen in the general population. The Magriples hypothesis remains unconfirmed by any other study. (Assikis and Jordan., 1995).

MacMahon (1997) concluded that published reports of a causal association between tamoxifen therapy and endometrial carcinoma were not conclusive for several reasons. Among these were 1) several randomized studies in which no increase in endometrial cancer was noted, 2) problems with the comparison groups in the three positive studies, 3) failure of the positive studies to deal with confounding factors and 4) the possibility of detection bias. An IARC Working Group acknowledged that most studies had not properly addressed several potential confounding factors. However, it concluded that potential confounders were unlikely to have a major effect on the reported relative risks. (IARC 1996). It is unclear on what basis the IARC Working Group reached this conclusion, since the IARC monograph dismissed the possible role of confounding factors in a single sentence.

Following publication of the IARC monograph dealing with tamoxifen, a case-control study done by Dr. Leslie Bernstein identified both obesity and prior use of unopposed estrogen replacement therapy as confounding the association of tamoxifen and endometrial cancer. Furthermore, the magnitude of the confounding effect was similar to that of the reported association of tamoxifen and endometrial cancer. These data were presented at the 13th International Conference of Pharmacoepidemiology (Orlando, FL August, 1997), and preliminary data were published in part in abstract form. (Bernstein, 1997). These data call into question the judgment by a working group of IARC that confounding factors were unlikely to have a major effect on the relative risks reported in the NSABP and Stockholm clinical trials and other human studies. If tamoxifen does interact with previous unopposed estrogen replacement therapy, the public health significance of this is likely to diminish, as use of unopposed estrogen replacement therapy is less common now than it was in the 1970's and early 1980's, current practice generally to use estrogen combined with progestins for hormone replacement therapy. (Creasman, personal communication).

As noted by a working group of IARC, experimental animal studies provide evidence of tamoxifen's carcinogenic effects in animals. However, the carcinogenic effect is highly tissue and species specific, and bring into question the validity of direct extrapolation of data generated in a single susceptible species, the rat, to women in assessing potential risks of tamoxifen administration. (Wogan, 1997). The benign ovarian and testicular tumors seen in mice exposed to tamoxifen reflect the pharmacologic action of tamoxifen in mice, and not carcinogenicity. The histopathologic changes seen in the uteri of rats exposed to tamoxifen differ from the effects of tamoxifen on the human uterus. Uterine squamous cell carcinomas in rats have only been reported in one study (Mantyla, 1996) that is at variance with numerous others.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenicity

The first paragraph of this section discusses both models in which tamoxifen inhibits carcinogenesis and models in which tamoxifen promotes carcinogenesis. The text is confusing, and should be modified to provide clarification.

The second paragraph of this section states that tamoxifen acts as an estrogen agonist in the human uterus. However, in section 7.2 of the draft RC document, tamoxifen is described as a partial agonist in the human uterus. (Love, et al., 1992; Jordan and Prestwich 1977). The latter is correct, and so the statement that tamoxifen "would likely produce the same effects as conjugated estrogens in the uterus" is wrong.

Several gynecologists have pointed out that the effects of estrogen and those of tamoxifen on the human uterus are very different. (Cohen, 1997; Creasman, 1997). Estrogens cause uniform hyperplasia of the endometrium. Tamoxifen causes endometrial atrophy with myometrial thickening, cystic glandular atrophy, and subendometrial edema. Although older studies using ultrasound showed what was thought to be endometrial hyperplasia in women treated with tamoxifen, this is now regarded as a false positive test, and represents stromal edema and cystic glandular atrophy.

The levels of DNA adducts found in human leukocytes and endometrial tissue by Hemminki (1996) are extremely low, and have not been reproduced by other investigators. (Carmichael, 1996 and Carmichael, 1997). Hence there are compelling data indicating that the mechanism by which tamoxifen induces hepatocellular carcinoma in rats does not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans. Furthermore, as stated earlier, although some studies have shown an increased risk of endometrial cancer in tamoxifen-treated women, detection biases and confounding factors raise substantial doubt as to whether causality has been established.

1.0 CHEMICAL PROPERTIES

No comments.

2.0 HUMAN EXPOSURE

2.1 Use

The statements, while generally correct, do not acknowledge the unique role of tamoxifen in the treatment of human breast cancer. "Tamoxifen, because of its efficacy and tolerability is the therapy of choice for postmenopausal women with advanced breast cancer. When used as adjuvant therapy, tamoxifen reduces the annual rates of both death from and recurrence of breast cancer by about 25%. Tamoxifen, has been widely adopted as the first-line therapy of choice for hormone-responsive male breast cancer and is frequently used as adjuvant therapy for estrogen receptor or progesterone-receptor positive male breast cancer (IARC, 1996)." Tamoxifen has been cited by the World Health Organization (of which IARC is a part) as an essential drug for the treatment of breast cancer. A review of 30,000 women who took part in clinical trials with tamoxifen as adjuvant treatment for breast cancer found tamoxifen treatment to be associated with a 25% decrease in recurrence and a 17% decrease in mortality ($2p<0.00001$) (EBCTCG, 1992). Tamoxifen is the world's leading hormonal treatment for breast cancer with over 7 million patient-years experience worldwide.

The statements about dosages refer to past practice. (IARC, 1996). At present, 20 mg daily for up to 5 years is a standard dosage worldwide for adjuvant use of tamoxifen, while for metastatic disease a dose of 20-40 mg daily is given until disease progression.

Tamoxifen is one of the most widely studied anticancer drugs. It continues to be investigated not only as treatment for breast cancer, but also for other cancers including endometrial cancer, melanoma, and primary hepatocellular carcinoma. Tamoxifen has not been cleared for marketing by the Food and Drug Administration for treatment of cancers other than breast cancer.

The draft document by NTP does not address the adverse effects on public health that might result from the listing of tamoxifen as a carcinogen. Such a listing could cause increased stress for patients and interfere with patient-physician communications thereby decreasing compliance not only with taking medications but also with other aspects of treatment. (Spiegel, 1997). Decreased compliance can in turn lead to unnecessary recurrences of breast cancer and years of life lost. It should be noted that tamoxifen product labeling is regulated by the Food and Drug Administration, and that appropriate actions to protect the public health have already been taken.

2.2 Production process and volume

No comments.

2.3 Environmental exposure

No comments.

2.4 Occupational exposure

No comments.

2.5 Regulations and criteria

Zeneca would propose that the first sentence read that tamoxifen was "cleared for marketing" in the U.S. in 1977 rather "allowed on the market."

Tamoxifen was listed as a carcinogen by California in September 1996, not May 1995. Zeneca provided comment on the proposed listing with an extensive written submissions, and oral testimony at the October 1995 public forum at which a number of experts provided comments. Zeneca provided comments as the scientific evidence as of that date did not clearly demonstrate tamoxifen to be a carcinogen in humans, the animal studies conducted did not provide a basis for predicting or assessing human carcinogenicity, and the proposed listing raised public health concerns. It should be noted that the Carcinogen Identification Committee of the State of California which proposed the listing had no mandate to consider the possible adverse public health impact of listing a widely used anticancer agent as a carcinogen, nor did they consider such an effect.

3.0 HUMAN STUDIES

Based on its review of the then available published literature, a working group of IARC concluded that there is sufficient evidence in humans for the carcinogenicity of tamoxifen in increasing the risk for endometrial cancer, and there is conclusive evidence that tamoxifen reduces the risk for contralateral breast cancer in women with a previous diagnosis of breast cancer. (IARC 1996). It found there is inadequate evidence in humans for the carcinogenicity of tamoxifen in other organs. (IARC 1996).

IARC did acknowledge the possibility of potential confounding factors, which had not been systematically addressed in most studies, but these were dismissed as unlikely to have had a major effect on the reported results. The IARC monograph does not discuss the basis for this decision. Ongoing work now confirms that obesity and prior estrogen replacement therapy do have an effect on endometrial cancer incidence comparable to the reported association of tamoxifen and endometrial cancer. These data were presented at the 13th International Conference on Pharmacoepidemiology held in Orlando, FL, August 1997. Partial, preliminary data also appears in abstract form. (Bernstein, 1997).

Conjugated estrogens have been listed as a carcinogen by NTP. (7th Annual Review on Carcinogenesis, 1994). Data on participants' prior use of estrogen replacement therapy are missing from most trials. (MacMahon, 1997). Since use of hormone replacement therapy to relieve menopausal symptoms has been widespread, it is reasonable to conclude that many women in these trials may have received estrogen replacement therapy prior to their development of breast cancer. Occult endometrial carcinomas are not uncommon, with reported incidences of 1.71 per 1000 women-years on screening of asymptomatic women (Koss, et al., 1984) and 2.2-3.1 per 1000 women at autopsy (Horwitz, et al., 1981). Among women treated with estrogens, the incidence of occult carcinomas could well be higher. As several noted epidemiologists have stated, the increased incidence of endometrial cancer seen among tamoxifen-treated women in several trials may have been due either to detection bias or to an "unmasking" effect. (MacMahon, 1997; Creasman, 1997).

Detection bias arises from the fact that patients taking tamoxifen are more likely than control patients to develop gynecologic symptoms, such as hot flushes or vaginal discharge. If they see a gynecologist for these symptoms, and diagnostic tests are performed, they may reveal a subclinical carcinoma which in and of itself was asymptomatic. The asymptomatic control patient will not undergo such investigations, and her cancer will not be detected at that time. Hence the close scrutiny of the tamoxifen-treated patients results in an apparent higher risk for the tamoxifen-treated patients, but this reported higher risk does not reflect the actual clinical situation. Since in the clinical trials cited, endometrial cancer was evaluated retrospectively, no provision was made in the trials for testing both the tamoxifen and the control patients for the presence of endometrial cancer with equal frequency. One study which did so is that of Lahti (1993) which found one endometrial adenocarcinoma in the tamoxifen-treated group and 2 endometrial adenocarcinomas in a control group matched for age, parity, body mass index, and age at menopause.

An unmasking effect can occur if a woman has previously taken a substance (such as conjugated estrogens) which can cause endometrial cancer. This woman may have an occult cancer at the time of entry into the clinical trial. (Note baseline tests for the presence of occult endometrial cancers were not done in the trials cited.) If she then takes tamoxifen and develops symptoms requiring gynecologic evaluation, the endometrial carcinoma, although not itself symptomatic, will be "unmasked." In such cases, the cancer would actually have been caused by an exposure which occurred prior to tamoxifen administration.

The National Surgical Adjuvant Breast and Bowel Program (NSABP) is currently conducting a trial under the auspices of the National Cancer Institute to assess the worth of tamoxifen in preventing breast cancer in women at high risk for development of breast cancer. This trial finished recruiting its full complement of patients September 30, 1997. Baseline endometrial biopsies were performed on all participants recruited after July 1994, and all patients recruited after September 1994 will have yearly gynecologic evaluations. Results from this trial will be available in 1-2 years, and will give the first prospective data on the occurrence of endometrial cancer in tamoxifen-treated patients compared with placebo-treated patients. This trial is designed to avoid the biases and confounding present in the trials considered by IARC. To list tamoxifen as a carcinogen in humans would therefore be premature.

Furthermore, several of the studies (Fisher et al., 1994; Rutqvist et al., 1995; Andersson et al., 1991) including the two which show a statistically significant risk in tamoxifen-treated patients, have a problem with an unexpectedly low incidence of endometrial cancer in the control group. (MacMahon, 1997). This would make the relative risks of 7.5 and 6.4 as cited in the draft NTP document excessively high. In NSABP B-14, endometrial cancer was diagnosed in 15 patients assigned to the tamoxifen group, and 2 patients assigned to the placebo group, giving a relative risk of 7.5. However, SEER data, suggested there should have been 6.9 endometrial cancers in the control group. Data previously obtained from NSABP B-06 also suggested that the incidence of endometrial cancer in the control arm of NSABP B-14 was unexpectedly low. When compared with SEER rates, the odds ratio for the tamoxifen-treated patients was only 2.2, while when compared with the rates in the B-06 trial the odds ratio was 2.3. (MacMahon, 1997).

Rutqvist reported 13 cases of endometrial cancer in the tamoxifen group, and 2 in the controls, for a relative risk of 6.4. If rate and age distribution are comparable to that of Fisher et al, one would expect 2.9 cases in the control group (MacMahon, 1997), with a corresponding reduction in relative risk.

Andersson et al. (1991) reported a cumulative 10-year incidence rate of endometrial cancer of 1.0% in the group which received tamoxifen and radiation therapy, compared with 0.3% in the group receiving radiation therapy alone. That the radiation therapy group has an unusually low number of cases is suggested by comparison with the untreated group, in which the incidence rate was 0.8%, almost indistinguishable from the tamoxifen group. The above examples provide evidence that the relative risks of 7.5 and 6.4 quoted in the NTP document are indeed excessively high as a result of an unusually low number of cases in the control groups. (MacMahon, 1997).

It is important to realize that breast cancer itself is a risk factor for developing endometrial cancer, so a comparison with the general female population is not appropriate. (relative risk 1.72, Adami, et al., 1987). Control groups must also have the same age, age at menopause, body weight, and presence of an intact uterus. Most of the trials mentioned in the NTP document did not adequately investigate these factors.

Zeneca concurs with the finding that there is insufficient evidence in humans for the carcinogenicity of tamoxifen in other organs. (IARC, 1996). The study by Rubagotti (Rubagotti et al., 1996) cited in the draft NTP document shows that tamoxifen-treated patients in fact had a lower incidence of second primary cancers than untreated patients, and that patients treated with chemotherapy plus tamoxifen had a lower incidence of second primary cancers than patients treated with chemotherapy alone. This was true even after second primary breast cancers and skin cancers were removed from the analysis. One notes that only 4 cases of endometrial cancer were seen in the study, 3 on tamoxifen arms and 1 on the no treatment arm. The danger in drawing conclusions from such small numbers is evident.

Most other studies of tamoxifen in early breast cancer have also shown a reduced incidence of second primary breast cancers in tamoxifen-treated women. In the largest studies, the difference was statistically significant. Fisher found a 37% reduction in contralateral breast cancer in tamoxifen-treated women ($p = 0.007$) (Fisher et al., 1996), while Rutqvist found a 40% decrease ($p = 0.008$), (Rutqvist, et al., 1995). In the metaanalysis done by the Early Breast Cancer Trialists Cooperative Group, a 39% reduction in contralateral breast cancer was found ($p < 0.00001$) (EBCTCG, 1992). As noted previously, IARC found there was conclusive evidence that tamoxifen reduces the risk for contralateral breast cancer in women with a previous diagnosis of breast cancer (IARC, 1996). It is unclear how a substance which reduces the risk of second primary cancers can properly be called a carcinogen.

4.0 EXPERIMENTAL CARCINOGENESIS

It has now been established that rats (Sprague-Dawley, Fisher and Wistar) develop liver cancers (hepatocarcinomas) after long term tamoxifen exposure, generally by oral dosing. Thus, Zeneca does not dispute the conclusion of an IARC working group that there is sufficient evidence for carcinogenicity of tamoxifen in animals.

However, the NTP draft RC document appears to suggest that IARC made this finding based in part upon the single study demonstrating benign ovarian and testicular cancers in mice. (Tucker, et al., 1984). This is misleading. The tumors reported in this study were benign, and consistent with the pharmacologic action of tamoxifen in mice. While it has been established that tamoxifen is a species-specific liver carcinogen in rats, in peer reviewed, published experimental studies exposing adult mice to tamoxifen, no increased incidence of malignant tumors of reproductive or any other organ has been established. The dose of tamoxifen used in the mouse study was similar in frequency, magnitude and duration to that which induced hepatocellular carcinomas in rats.

More specifically, although some epidemiologic data from patient studies report an increased incidence of endometrial cancers associated with tamoxifen treatment (See discussion above), no similar effect has been demonstrated in animal carcinogenicity studies. Appropriately conducted rat and mouse preclinical studies have reported no association at all between tamoxifen and uterine tumors.

The only two studies reporting reproductive tract tumors in rodents employed non-standard protocols. The first (Newbould et al., 1996; 1997), exposed male and female mice not as adults but in the early days of neonatal life. An increased incidence of tumors was observed in each sex but the administration of tamoxifen occurred during the first five days of life, a time in which the reproductive tract in mice is undergoing rapid maturation. No comparable changes were studied or reported in adult animals. Thus, not only does the study employ a non-standard protocol for carcinogen testing, it is inapposite to the human clinical dosing situation. In humans, the corresponding period of reproductive tract maturation occurs during mid-pregnancy, and tamoxifen treatment is strictly contraindicated for pregnant women.

The second study reported increases in well differentiated squamous cell metaplasia of the uterus, as well as a few squamous carcinomas in rats. (Mantyla, et al., 1996). The study has not been reproduced and validated and its data present a number of problems, including the indication that more animals were tested at the high dose than at the low dose or any of the groups tested for the comparison substance (toremifene). Tamoxifen was also withdrawn for 13 weeks before analysis. The study is inconsistent with a series of studies which have indicated that tamoxifen may in fact inhibit hyperplasia and carcinogenesis induced by estrogenic substances in the reproductive tract and other organs in various species, including mice, rats and hamsters. (Segupta, et al., 1991; Yager, et al., 1986; Kohigashi, et al., 1988; Liehr et al., 1988; Coe, et al., 1992). While the authors speculate as to a genotoxic mechanism, this theory is inconsistent with the absence of DNA adducts observed in rat uteri. (See below) The study findings are also inconsistent with numerous examples of decreased uterine weights observed in tamoxifen-treated rats, which suggest an anti-estrogenic effect on the uterus in rats. (Greaves, et al., 1993; Hard, et al., 1993; Hirsimaki, et al., 1993; Dragan, et al., 1994).

In summary, the rat is not a good predictor of the human experience. In experimental studies, tamoxifen is a species specific liver carcinogen inducing tumors in the rat, but not in the mouse or other species. (IARC 1996). Tamoxifen exposure does not appear to lead to endometrial carcinoma nor to DNA adducts in the uterine tissue of rats.. As stated by Wogan (1977), critical examination of the evidence indicates that extrapolation of experimental data to humans is subject to very substantial uncertainty.

5.0 GENOTOXICITY

While Zeneca does not take issue with the assessment of an IARC working group that tamoxifen is a genotoxic liver carcinogen in the rat, accumulated evidence would indicate that the human liver is not susceptible. The mechanism of tumor induction in rat liver most likely involves the formation of DNA reactive metabolites (Phillips, et al. 1996) and the replication of initiated hepatocytes (Carthew, et al., 1995; 1996).

As recognized in the draft RC document (p. 5-1), tamoxifen has been reported negative for DNA adduct formation in primary human hepatocytes. Thus although DNA adducts have been widely reported in the liver tissue of rats treated with tamoxifen, this does not appear to be predictive of any mechanism relevant to the human clinical experience.

Additionally, there is no evidence that tamoxifen exerts a genotoxic effect on the human endometrium. In fact, there is no evidence that tamoxifen exerts a genotoxic effect on the uterus of any species.

> Point No.1 No tamoxifen DNA adducts or genotoxicity have been observed in the uteri of rats and mice (as summarized in IARC, 1996; Davies, et al., 1997).

Point No.2 Until late 1996, the only study examining tamoxifen DNA adducts in the uterus of tamoxifen-treated breast cancer patients reported no adducts. (Carmichael, et al., 1996). The draft RC document cites a second study. (Hemminki, et al., 1996) as contrary evidence, suggesting that DNA adducts have now been reported in human endometrium. Hemminki and colleagues acknowledge that studies prior to theirs found no tamoxifen-induced DNA adducts in humans. Further the authors openly admitted that their samples contained blood and stromal cells, so that the adducts *could not be attributed to endometrium with any certainty*. The authors conclusions are then clearly stated "assuming that the adducts are indeed derived from endometrial cell DNA." (Hemminki, et al., 1996).

The methodologic flaws in this study have been criticized. (Orton and Topham, 1997). Those criticisms have more recently been confirmed by other authors. (Carmichael, et al., 1997). In addition to the uncertain origin of the adducts it is noteworthy that:

- The sample size was small -- only 11 patients (6 tamoxifen patients, 5 controls).

- The sample was poorly matched. Although fairly close in median age (TAM 67, control 59), all tamoxifen-treated women obviously had breast cancer, whereas only 2 of the 5 controls had breast cancer. This is significant because breast cancer itself is an acknowledged risk factor for endometrial cancer.

- The study involved women of perimenopausal or menopausal age, whose aging could alone account for spontaneous DNA adducts or endometrial cancer. Moreover, the baseline level of adducts drifts.

- For no explained reason, the authors obtained markedly different results on HPLC gradient A and gradient B; for the latter, the adduct levels were very similar to the controls. Perhaps for this and the foregoing reasons the authors choose the lower increase to report as the "apparent level" of adducts associated with tamoxifen.

- Even if the adducts were attributable to endometrium, the study sample may have been a poor predictor of tamoxifen exposure in the United States, where the generally used dose is 20mg. Of the tamoxifen patients, the majority (4 of 6) were under a 40mg treatment regime; only 2 received 20mg.

Therefore, the significance of the adducts reported in Hemminki, et al. (1996), and whether they have anything at all to say about the mechanism of tamoxifen action in the human uterus, are at best, questionable. The study results have not been replicated (Carmichael, et al., 1997), and in a separate paper Hemminki and coworkers repeat their acknowledgment of the uncertainty of their 1996 data, stating that the role of DNA adducts in tamoxifen treatment can be tested only "if and when adducts can be reliably measured in endometrial samples." (Hemminki, et al., 1997).

Point No. 3 The draft RC document reports that DNA adducts have been reported in one study of human leukocytes. (Hemminki, et al., 1997). The significance of these adducts, and whether they have anything at all to say about the mechanism of tamoxifen action in the human (particularly in the uterus) is, again, unclear.

Studies prior to Hemminki, et al. (1997) found no tamoxifen-induced DNA adducts in humans, including human leukocytes. This is acknowledged by the authors. (Hemminki, et al., 1996). The Hemminki results have not been replicated and the study has been subject to criticism for its methodologic flaws. (Orton and Topham, 1997). For instance:

- The sample size was again small -- only 11 patients (6 tamoxifen patients, 5 controls).

- The sample was poorly age-matched. The median age of tamoxifen patients was 72, whereas the median age of controls was 51. Clearly circulating estrogen was likely to differ in the two groups. Differences in aging or accumulated environmentally-related spontaneous adduct formation were likely.

Clearly the presence of detectable DNA adducts alone does not predict carcinogenicity in tamoxifen-treated species. As the draft RC document reports, DNA adducts have been identified in the livers of mice and Syrian hamsters exposed to tamoxifen. Yet these species do not develop tamoxifen-associated liver cancer. As the document further reports, DNA adducts have been identified in the lungs and kidneys of mice and in the kidney of rats exposed to tamoxifen. Yet despite these findings, experimental carcinogenicity studies have not found carcinogenicity in these rodent organs.

Point No.4 Zeneca recognizes that tamoxifen has a demonstrated genotoxic effect on various cells in various in vitro tests and animal-based mutagenicity and clastogenicity assays (summarized in Tannenbaum, 1997). However, it should be clear that genotoxicity does not equate to carcinogenicity. For instance, chromosome aberrations were observed in the mouse (Vijayalaxmi and Rai, 1997), a species in which tamoxifen does not induce malignant tumors. Tamoxifen shows little activity in *vitro* assays, particularly in normal human cells. (Tannenbaum, 1997). The poor predictive value of micronuclei, in particular, should be self evident, as tamoxifen has been reported to increase micronuclei in human breast cancer cells which it is used to treat.

6.0 ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

6.1 Absorption, distribution and excretion

No comments.

6.2 Metabolites

The draft RC document recognizes that there now appear to be at least two different pathways of tamoxifen metabolism, and a working group of IARC has schematically presented postulated metabolic pathways.
It is important to recognize that the different pathways are hypothesized to lead to metabolites of differing reactivity and ability to form DNA adducts. The DNA adducts formed from the alpha-hydroxy pathway may differ from those formed by 4-hydroxytamoxifen.

The metabolic pathways may not be identical species-to-species or organ-to-organ. Different species may also have different capacities to detoxify or clear various metabolites.
Caution should be used when interpreting the literature using enzymes, microsomal preparations and genetically engineered cells, when these experimental systems lack various detoxifying enzymes. (Tannenbaum, 1997).

Point No.1 A quantitative, if not qualitative, difference in tamoxifen metabolites appears to exist between humans and rats.

- The major differences between rat and human liver microsomes reported in one study were in the lower amount of hydroxylated metabolites and trace amounts of several other metabolites detected in humans. (Lim, et al., 1994).

- Five different analyses have shown that two epoxides, 3,4-epoxytamoxifen and 3'4'-epoxytamoxifen are formed by rat liver microsomes, while only the former was detected in mouse and human microsomes. (Lim, et al., 1994).

- One study using *in vitro* incubations of liver microsomes demonstrated the same metabolites formed by human liver microsomes as rat liver, but the rate of metabolism was markedly lower than in the rat. (Mani, et al., 1993; 1994). Other authors have reported that tamoxifen appears to be more slowly metabolized in humans than in rats. (Lim, et al., 1994).

- For humans, the range of 4-hydroxytamoxifen in liver tissue has been reported at nearly 1/10th that seen in rat liver tissue during steady state treatment. Similar differences were seen for other tamoxifen metabolites. (Lien, et al., 1991).

Point No.2 Liver concentrations of tamoxifen and its metabolites may be significantly higher than plasma levels or concentrations in other organs.

- Rat liver concentrations of tamoxifen have been reported as 20-fold to 30-fold higher than serum concentrations. Liver concentrations of tamoxifen metabolites in rats have been reported as 100-fold higher than serum metabolite levels. (Dragen, et al., 1994; Carthew, et al., 1995a and b).

- As recognized by NTP, similar differences have been reported in humans for biopsy and autopsy samples of liver and lung tissue as compared to serum. (Lien, et al., 1991).

6.3 Structure-Activity Relationships

The draft RC document examines toremifene for this analysis. This would seem a scientifically inappropriate method for a Structure-Activity evaluation. The pharmaceutical, toremifene, has only a slight database testing its carcinogenicity. The clinical (human) experience with Toremifene is even more limited. IARC (1996) recognized the undeveloped state of research regarding toremifene, and therefore concluded that toremifene was not classifiable. There has been even less clinical experience with droloxifene and 4-iodotamoxifen.

One should note that toremifene causes chromosome damage in the human MCL-5 cell line. Toremifene has also been reported to cause osteosarcomas in mice. (Hayes, et al., 1995). Hence the presence of a chlorine substituent may not render toremifene free of carcinogenic potential.

A comparison of the effects of tamoxifen vs. other antiestrogens in humans can only be done in the context of a randomized, double-blind head-to-head trial. Conjecture about structure-activity relationships will not answer the question. Trials of tamoxifen vs. toremifene in postmenopausal women with advance breast cancer show the two drugs to have similar side effect profiles. (Hayes, et al., 1995). Histologic effects on the uterus were also similar. (Tomas, et al., 1996). Cases of endometrial cancer have been reported in patients who have taken toremifene. (FDA 1997).

7.0 MECHANISMS

7.1 Genotoxicity

The draft RC document states (para. 1) that a possible mechanism by which tamoxifen is carcinogenic is via formation of DNA adducts induced by one or more genotoxic mechanisms. It also broadly states that some studies support a causal relationship between in vivo genotoxicity and tumor response (para. 2). These statements should be strictly restricted to rat liver cancer. The absence of tumors in mice where adduct levels are still substantial illustrates that tumor formation requires both mutation of DNA and promotion of mutant cells via replication as occurs in the rat. Humans are known to be less susceptible to the hyperplasia induced by a wide range of agents in rodents (Grisham, 1996).

As discussed above, while the relationship between DNA adduct formation and liver cancer in the rat appears likely, evidence does *not* support a causal relationship between *in vivo* genotoxicity and tumor response *in humans*.

As noted above, a quantitative, if not qualitative difference in tamoxifen metabolites may exist between humans and rats, and between various organs. Tamoxifen-protein binding is greater in liver microsomes of the rat (and also mice) than of humans. (White, et al., 1993; Smith and White, 1995). It remains true that the relationship between the adducts formed in vitro and the DNA modifications formed in vitro is poorly understood, as it is not known which gene is mutated and which of the 10-12 adducts is responsible. (Moorthy, et al., 1996).

As noted by one reviewer, "major differences between women and rats with respect to the activation of tamoxifen and formation of DNA adducts ... bring into question the validity of direct extrapolation of data generated in the single susceptible species, the rat, to women in assessing potential risks attendant to tamoxifen administration." (Wogan, 1997).

7.2 Tamoxifen-estrogen receptor interaction

As the draft RC document recognizes, tamoxifen acts as a partial agonist -- i.e. not as an estrogen equivalent -- in various organs, including the uterus. The distinction between the effects of tamoxifen and the effects of estrogen is essential to understanding whether tamoxifen causes cancer in the human uterus through an estrogen-like mechanism.

Point No.1 Metabolic differences in association and dissociation rates have been confirmed between tamoxifen and estrogen, as well as between tamoxifen and its various metabolites. (studies summarized in Cohen, 1997).

Point No.2 Experimental studies examining the effect of tamoxifen and the effect of estradiol on endometrial cancer transplanted into experimental rodents similarly reveal that tamoxifen does not act as an estrogen equivalent. (studies summarized in Cohen, 1997).

Point No.3 The clinical effect of tamoxifen on the human uterus does not mimic that of estrogen. Tamoxifen is associated with clinical evidence of atrophy, which is not what would be expected with an estrogenic effect. (studies summarized in Cohen, 1997).

Point No.4 The actual effect of tamoxifen on the uterus is not the uniform, hyperplastic endometrial changes that one sees when a patient is exposed to estrogen. Physicians have long relied on a widened endometrial stripe as sonographic evidence of endometrial cancer. However, it is now clear that, in the case of tamoxifen-treated patients, such an interpretation leads to false positives in diagnosis of endometrial hyperplasia or endometrial cancer. Unlike the situation with estrogen treatment, histologic follow up of ultrasound on tamoxifen-treated women reveals that the changes are *not* attributable to changes in the endometrial epithelium. Rather, they often are attributable to collagenous changes in the stromal layer, increased secretory fluid inside dilated glands, hyperplasia of the myometrium or increased blood perfusion. (studies summarized in Cohen, 1997).

Point No.5 Although several reports suggest that tamoxifen patients have a higher rate of endometrial polyps, hyperplasia and other proliferations, "there is considerable evidence to suggest that an etiologic relationship between these features and endometrial cancer has not been demonstrated." First, many of these reports rely on ultrasound for their conclusions. Second, autopsy data have found that 10% of uteri contained endometrial polyps, a rate similar to the incidence found in tamoxifen patients. (studies summarized in Cohen, 1997).

Point No.6 Evidence that tamoxifen is not an estrogen equivalent in the uterus is also apparent in the fact that tamoxifen has been used successfully for the treatment of endometrial cancer and pre-cancerous proliferative disorders of the endometrium such as endometrial hyperplasia. Studies by one author have reported "a consistent 20 to 30 percent response rate in patients with advanced recurrent endometrial cancer."

(Swenerton, et al., 1984). Similar results have been reported in other studies. (studies summarized in Cohen, 1997). The investigators in the Stockholm trial acknowledged that "the clinical observation of an increased risk of endometrial cancer seems like a paradox" in light of the fact that tamoxifen is useful treatment for endometrial cancer. (Fornander, et al., 1991).

Point No.7 In several studies, asymptomatic breast cancer patients demonstrated no aggravation of any preexisting endometrial lesions after treatment with tamoxifen continuously for 18 months. (Cohen, et al., 1994).

Point No.8 Further evidence that tamoxifen does not act as an estrogen equivalent is apparent from the fact that it had been used for the treatment of other cancers without a reported increase of endometrial or estrogen-related cancers in these populations. (studies summarized in Cohen, 1997 and Gelman, 1997).

Point No.9 The diagnosis of endometrial cancer is usually made because the patient presents with abnormal genital bleeding. It is well recognized that endometrial cancer may be occult and asymptomatic, and its finding is merely fortuitous. Autopsy studies have identified an occult endometrial cancer rate of between 2.2-3.1 per 1,000. (studies cited by Creasman, 1997). Although IARC dismisses this data, in the two major clinical trials, endometrial cancers have been reported to be associated with tamoxifen use when they were more likely pre-existing and occult. The latency period from onset of treatment was far too short to support a causal relationship. (Fisher, et al., 1994 [Tables 3 and 4 and comment p. 535]; Fornander et al., 1993, [Table 2, and comment p. 1853])

These data, for the most part overlooked by an IARC working group (IARC 1996), help to elucidate why tamoxifen may *not* be the biological cause of endometrial cancers reported, albeit in increased numbers, in tamoxifen-treated breast cancer patients. More importantly, this information, together with new and emerging clinical data regarding the background rate of endometrial cancers in breast cancer patients and the effect of other risk factors, urge that caution is well advised before concluding that an estrogen-like effect on the human uterus mediates the causation of endometrial cancers.

8.0 CONCLUSION

In 1994 and 1995, the NTP held a series of public meetings to consider the criteria for listing or delisting an agent, substance, or mixture in its *Biennial Report on Carcinogens.* As a result of those meetings, the NTP adopted a new view that places greater emphasis on the NTP assuring that it recognizes advances in understanding the biological events involved in carcinogenesis and a fuller consideration of risk-benefit factors.

Zeneca wishes to highlight to the NTP Board of Scientific Counselors that there is insufficient proof of causality between tamoxifen and endometrial cancer. Zeneca believes that the new data on tamoxifen which have emerged from the study by Dr. Leslie Bernstein and the additional definitive results on tamoxifen and endometrial cancer which will emerge in one to two years from the NSABP P-1 trial, which is being conducted under the auspices of the NCI, will offer critical information in understanding the important role of confounding factors concerning tamoxifen's association with endometrial cancer. These critical new data, which were not available at the time IARC reviewed tamoxifen, coupled with the public health risk of unnecessarily alarming breast cancer patients who are taking tamoxifen, create a considerable imperative not to include tamoxifen in the NTP's *Biennial Report on Carcinogens*.

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